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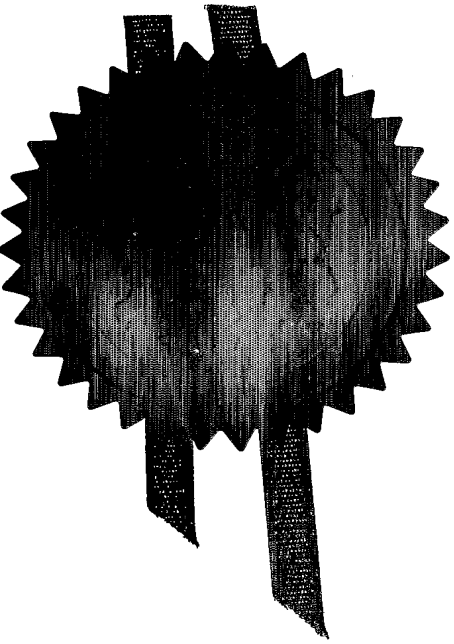
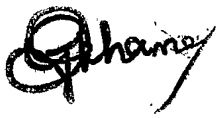


Signed 

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1. **Your reference** 59.83822

2. **Patent application number** *(The Patent Office will fill in this part)* 0406728.6 — 25 MAR 2004

3. **Full name, address and postcode of the or of each applicant** *(underline all surnames)*
Hydrodynamic Gene Delivery Ltd
21 Wilson Street
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EC2M 2TD

25MAR04 E883942-1 D00027
P01/7700 0.00-0406728.6 NONE

Patents ADP number *(if you know it)* 8837171001

If the applicant is a corporate body, give country/state of its incorporation United Kingdom

4. **Title of the invention** Gene Therapy

5. **Name of your agent** *(if you have one)* Frank B. Dehn & Co.

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*
179 Queen Victoria Street
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Patents ADP number *(if you know it)* 166001

6. **Priority: Complete this section if you are declaring priority from one or more earlier patent applications, filed in the last 12 months**

Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*

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8. **Is a Patents Form 7/77 (Statement of inventorship and of right to grant of a patent) required in support of this request?**
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9. Accompanying documents: A patent application must include a description of the invention. Not counting duplicates, please enter the number of pages of each item accompanying this form:

Continuation sheets of this form	
Description	14
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Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*	
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11. I/We request the grant of a patent on the basis of this application.

Signature Frank B Dehn & Co. Date 25 March 2004

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Rebecca Gardner
01273 244200

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Gene Therapy

The present invention relates to apparatus and methods for the introduction of nucleic acid into a target organ of the human or non-human animal body, in particular into the liver.

While gene therapy is of tremendous potential benefit in the treatment of hereditary and acquired diseases, one of the main hurdles to current gene therapy techniques is the low level of transfection which is seen in the clinics. Gene therapy relies on the animal cells taking up the vector which incorporates the therapeutic nucleic acid as transfection is necessarily a prerequisite to efficient gene expression. Even if the administered nucleic acid is a regulatory rather than a coding sequence it must still be taken up by the cell in order to exert its influence on the cell's protein production.

The central role which the liver plays in the body in terms of protein production and the prevalence of liver cancers makes this organ a key target for gene therapy. However, systemic injection, for example into the vein of the arm (vena mediana cubiti), has not resulted in significant transfection of the liver hepatocytes (Habib et al., Human Gene Therapy 12: 219-226 [2001]). The hepatocytes are the major cell type of the liver and they are responsible for the synthesis, degeneration and storage of a wide range of substances including the synthesis of all plasma proteins except for antibody and transfection of these cells must be achieved if the therapy proposed relates to any normal liver function.

Attempts have been made to make local injection into the hepatic artery (Habib et al. supra and Reid et al., Cancer Research 62: 6070-6079 [2002]) but again the

transfection rate of the hepatocytes was highly unsatisfactory.

It has often been proposed to inject tumours directly when the proposed therapy is cancer therapy; however most cancers that recur after surgery or radiation are multifocal and therefore intratumoral injection is not feasible in these circumstances.

Therefore a need exists for new ways of transfecting liver cells at efficiencies which can result in effective gene therapy.

In mice hydrodynamic injection has been used to transfect liver cells (Liu et al., Gene Therapy 6: 1258-1266 [1999]). In this case a large volume of fluid containing the plasmid vector encoding the gene of interest was injected forcefully and fast into the tail vein of the animal. The volume used is 1-2 ml which is equivalent to the total circulation in mice. The formulation of plasmid in saline goes up the vena cava up to the heart. The mouse heart does not cope with this volume and that forces the liquid carrying the plasmid to enter the hepatic veins of the liver. According to this technique, the pressure applied resulted in successful uptake of the plasmid by the hepatocytes due to the fluidity of the cell membrane.

However, such a technique would not be applicable to larger animals such as man where the forceful injection of large volumes of fluid into the systemic circulation would lead to heart failure.

The present invention addresses these problems and utilises hydrodynamic principles to achieve transfection of liver cells at levels adequate for gene therapy without damaging the heart.

Thus, in one aspect, the present invention provides a method for introducing nucleic acid into liver cells, which method comprises substantially occluding a hepatic vein and introducing said nucleic acid into the liver under pressure via said hepatic vein.

In a further aspect the invention provides apparatus for introducing nucleic acid into liver cells comprising: a reservoir for holding a liquid formulation which comprises said nucleic acid; a catheter tube in fluid communication with said reservoir for conveying said liquid formulation to the liver of a patient via a hepatic vein; pressure development means for pressurising the liquid conveyed by the catheter; and occlusion means for substantially occluding said hepatic vein.

According to the normal circulatory system blood enters the liver from the hepatic artery and hepatic portal vein and is then collected in one of three hepatic veins (right, middle and left) and travels from there to the heart. Thus by substantially occluding one of the hepatic veins the liver may be temporarily and partially isolated from the normal circulation. Importantly, the effect of the occlusion means is that when the liquid formulation comprising the nucleic acid with which it is desired to transfect the liver cells is introduced into the liver under high pressure, the heart is substantially isolated from this liquid. This means the heart cannot be damaged by exposure to high-pressure liquids and means the pressure at the site of delivery in the liver is such that uptake by the liver cells of the nucleic acid is sufficient to allow successful gene therapy. Occlusion of and delivery through the left hepatic vein is preferred according to the present invention.

The occlusion means could take any suitable known form. It is for example envisaged that a mechanical expansion mechanism, e.g. umbrella style could be employed. However preferably the occlusion means comprises a balloon arranged to expand to conform to the vein wall, e.g. upon being filled with fluid, preferably saline. The occlusion means could be provided separately of the catheter tube but is preferably provided integrally thereon.

In some embodiments two or more occlusion means could be provided. This would allow, for example, substantial isolation of the hepatic vein both upstream and downstream of the point of introduction of the nucleic acid.

The pressure development means could take any convenient form but is preferably operatively associated with the reservoir in order to pressurise the liquid formulation to a predetermined pressure. In a simple convenient example the reservoir comprises an ordinary syringe and the pressure development means an ordinary syringe driver. The syringe driver may then be programmed to deliver the liquid formulation at a predetermined rate which will determine the pressure at which the formulation is administered to the liver for a given catheter lumen bore, aperture size etc. Of course more complicated arrangements are also envisaged which could include for example a pressure sensor to form a feedback loop. The reservoir may comprise a flexible bag, as used in a saline drip for example, which may be provided with a jacket by way of pressure development means which can expel the liquid formulation in a controlled manner.

Preferably the pressure development means is adapted to allow delivery of the formulation comprising the nucleic acid to the liver under a pressure which is sufficient to cause uptake by the liver cells of the nucleic acid. Suitable pressures include 10 - 80 mmHg for example 15 - 50 mmHg, preferred pressures include 20 - 60 or 30 - 50 mmHg.

The catheter may be arranged to introduce the nucleic acid into the vein substantially axially, substantially radially, at an intermediate angle or any combination thereof. Radial introduction is presently preferred since this allows occlusion means to be provided on the catheter both up and downstream of the point of introduction, thereby allowing the introduction

site to be substantially fully isolated and unaffected by normal blood flow.

Preferably the degree of transfection is enhanced by the use of ultrasound. The source of ultrasound may be external to the animal being treated but preferably application of ultrasound is localised particularly by placing the source within the liver and preferably by incorporation into the catheter. Thus in some preferred embodiments the catheter is provided with an ultrasonic oscillator arranged to generate ultrasonic vibrations in the region of nucleic acid delivery. The catheter may for example be provided with a piezo-electric transducer or an array thereof. The ultrasonic oscillator is preferably arranged to generate a directional oscillation so as to allow it to be directed at the targeted liver cells, thus minimising the power required.

The above apparatus is suitable for all types of gene therapy and thus the nucleic acid with which it is desired to transfect the liver cells may be in the form of or may comprise any of the vectors suitable for delivery of nucleic acid to a cell *in vivo*. Suitable vectors may simply be naked nucleic acid or liposomes which encapsulate nucleic acid. Naked nucleic acid, e.g. in the form of a plasmid, is particularly suitable for transfection of cells and is preferred for use according to the present invention. Plasmids based on the test plasmid used by Liu et al. supra are suitable and as shown by Liu et al. liver specific promoters are not required but may be used to increase specificity of gene expression.

More complicated but equally suitable vectors for delivery of nucleic acid to the liver and thus for transfection of the liver cells are viral vectors. Viruses are very well suited for use in gene therapy since foreign or heterologous genes or coding sequences may be inserted into the viral genome. After infection of the cell by the virus, the foreign nucleic acid is

delivered to the nucleus of the cell. While viruses are able to actively infect cells, the present method of hydrodynamic nucleic acid delivery results in a significantly increased "infection" rate and thus in effect an increase in the transfection rate and in the efficacy of the gene therapy. There are at least five classes of clinically available viral vectors, derived from (onco)retrovirus, lentivirus, adenovirus, adeno-associated virus and herpes virus. Those viral vectors whose genomes are integrated into the host cell DNA (oncoretroviruses and lentiviruses) may be preferred where stable genetic alteration in dividing cells is required. The other viruses mentioned persist in the cell nucleus as extrachromosomal episomes but are capable of mediating persistent transgene expression in non-proliferating cells. The most appropriate vector will depend on the particular gene therapy being attempted.

For convenience, the term "gene" is used herein to describe regions of nucleic acid not only that are transcribed into mRNA and translated into polypeptides (structural genes), but also those that are transcribed into RNA (e.g. rRNA, tRNA) and those that function as regulators of the expression of the former two types. Preferably the nucleic acid delivered to the liver will encode a structural gene relevant (directly or indirectly) to treatment of a given medical condition but it may be appropriate to introduce regulatory regions which, in combination with the genes already present in the cell, can provide a therapeutic benefit.

The nucleic acid molecule of the vector is typically DNA but may, for example where the vector is an RNA virus, be RNA. Antisense molecules, iRNA and siRNA may be suitable for certain therapeutic regimen. Non-viral vectors may contain cDNA and the nucleic acid may be linear or circular, e.g. as with plasmid DNA. DNA may be single or double stranded.

Where the nucleic acid encodes a protein which it is desired to express in transfected cells, the nucleic acid molecule will typically also comprise an operably linked promoter and possibly other regulatory sequences. For certain vectors, in particular viral vectors, the nucleic acid will also encode structural and other proteins involved with the generation of further vectors which can go on to transfect other cells, e.g. the gag, pol and env genes of an adenovirus. The design and construction of expression vectors being familiar to be skilled man and well described in the literature.

As in the methods described by Liu et al. supra, the present methods and the apparatus for use in such methods can be considered hydrodynamic methods of nucleic acid delivery. In other words a comparatively large volume of a liquid formulation containing nucleic acid (e.g. a DNA solution) is introduced rapidly into the vein. Thus, for humans and other animals of a similar size, between 100 ml and 1300 ml of liquid formulation may be introduced in a single, continuous or substantially continuous, administration. Volumes will depend on the age, sex and strength of the subject, for example a healthy young male may receive 800-1300 ml while an elderly woman should receive 200-600 ml.

The liquid formulation may comprise, in addition to the plasmid or other vector, any physiologically acceptable carrier, saline being particularly preferred. The concentration of the nucleic acid delivered will vary depending on the therapy proposed and may readily be optimised by the skilled man. Suitable dosages include between 5 and 50 mg, e.g. 10-30 mg of plasmid per 500 ml of saline; a typical dose suitable for most patients would be 20 mg of plasmid in 500 ml of saline.

The speed of injection will depend on the pressure to be generated. Typically, using a syringe based system, 500 ml of saline containing plasmid would be administered over 1/2-8, e.g. 1-3 mins. Clearly larger

volumes would generally require more time but more important than delivery time is the pressure at which the nucleic acid is delivered. In a closed fluid system as described herein, the pressure as monitored in the reservoir will correspond to the pressure at the point of delivery in the liver.

After rapid delivery of the nucleic acid, the hepatic vein is typically maintained in its occluded state for between 2 and 20, preferably 5-15, e.g. around 10 minutes. Reduction in occlusion is preferably achieved gradually, e.g. by slow deflation of the balloon.

According to a further aspect the present invention provides the use of a nucleic acid molecule in the manufacture of a medicament for introduction into the liver of a subject, under pressure, and via a substantially occluded hepatic vein. Suitable medicaments are described above and will typically consist, consist essentially of or comprise saline. As discussed herein, the nucleic acid may be naked, e.g. a plasmid or contained with a liposomal, viral or other vector. The nucleic acid and thus the medicament containing it are introduced for the purpose of performing gene therapy on the subject, specifically for transfecting liver cells within the subject. There are many specific therapies that may be performed in this way, including treatment of cancer, liver cirrhosis and other liver diseases as well as conditions which are not manifested within the liver but may benefit from the generation in the liver of proteins encoded by the nucleic acid with which the liver cells are transfected.

Certain preferred embodiments of the invention will now be described, by way of example only, with reference to the accompanying drawings in which:

Fig. 1 is a perspective view of a catheter in accordance with the invention and associated guide wire;

Fig. 2 is a sectional view through the catheter of Fig. 1;

Fig. 3 is a view similar to Fig. 2 of a slightly different embodiment;

Fig. 4 is a view similar to Fig. 2 showing the balloon inflated;

Figs 5a to 5c are schematic sectional views at varying levels of magnification showing the catheter being used;

Fig. 6a is a view similar to Fig. 4 showing the pressurised introduction of nucleic acid (conveniently represented throughout as circularised);

Fig. 6b comprises a series of three schematic sectional views of transfection of a liver cell; and

Fig. 7 is a sectional view through a catheter in accordance with another embodiment of the invention.

Turning firstly to Fig. 1 there may be seen a catheter 2 in accordance with an embodiment of the invention having a corresponding guide wire 4 passing axially therethrough. The catheter 2 generally comprises an outer housing 6 which is divided longitudinally by an inflatable balloon 8. In the uninflated state shown in Figure 1, the catheter and balloon is able to pass easily through the inferior vena cava via the heart and ascending vena cava.

A marker band 10 is provided around the foremost body section 6 in order to aid location in the body. The material of the marker band 10 will therefore depend upon the imaging system used.

Fig. 2 shows the catheter 2 in greater detail, with the guide wire omitted for clarity. It will be seen from this that the catheter 2 comprises two coaxial lumens 12, 14. The central lumen 12 opens out at the tip 16 of the catheter and in use receives the guide wire. The outer lumen 14 communicates with the interior of the balloon 8 by means of a circumferentially spaced series of apertures 18. The balloon 8 may therefore be inflated

and deflated by introducing and withdrawing saline from the outer lumen 14. The skin of the balloon 8 is elastic and can be inflated up to a diameter of up to around 18 mm for an adult human, around 9 mm for a child depending upon the volume of saline inserted. This is larger than the diameter of the hepatic vein where the catheter will be used. Fig. 4 shows a perspective view of the balloon 8 in its inflated state.

Fig. 3 is a view similar to Fig. 2 showing a slightly different embodiment. This embodiment differs from that of Fig. 2 only in that the balloon 8' is longitudinally extended as compared to the balloon 8 in Fig. 2. This may be advantageous in some circumstances as it will clearly have a greater area of contact with the vein wall and thus withstand a greater pressure without slipping for a given degree of inflation.

Use of the catheter described above in a method in accordance with the invention will now be described with additional reference to Figures 5a-5c and 6a-6b. Referring initially to Figures 1, 2 and 5a, the guide wire 4 is inserted into the inferior vena cava 20 by means of an introducer 22 and then through the heart 24 into the ascending vena cava 26 and into the right hepatic vein 28. The catheter 2 is then slid over the guide wire until the tip 16 thereof is located in the desired position in the hepatic vein 28. This may be achieved for example by monitoring the progress of the marker band 10 towards the tip of the catheter using an ultrasound or other suitable imaging system.

Once the tip 16 of the catheter is in position, saline is pumped into the outer lumen 14 in order to inflate the balloon 8 until it presses against the walls of the hepatic vein 28 which may be seen in Figure 5b. This fixes the location of catheter 2 in the vein and occludes the flow of blood to the heart 24. The guide wire 4 may then be fully or partly withdrawn. Thereafter a liquid formulation containing nucleic acid material for

the required gene therapy is injected through the central lumen 12 of the catheter at a controlled pressure. In this embodiment the required pressure is achieved using a pre-programmed syringe driver although many suitable ways of achieving this may be envisaged.

The ejection of the schematically-depicted nucleic acid 30 is shown in Figs. 5c and 6a. The occlusion of the hepatic vein 28 by the catheter balloon 8 retains the nucleic acid 30 at pressure within the liver rather than allowing it to travel up the ascending vena cava 26 to the heart 24. In a particular example the nucleic acid is introduced at a pressure of approximately 50 mmHg which pressure is withstood by the action of the balloon 8 on the walls of the vein 28.

The effect of this pressurised nucleic acid on the liver cells 32 in this area of the liver is to force the nucleic acid 30 through the walls 34 of the liver cells as is shown schematically in Fig. 6b, which then means that the nucleic acid is taken up by the cell 32 thereby allowing the nucleic acid to exert its influence on the cell's protein production.

In one example, the therapy is continued in this manner for up to 10 minutes, preferably 1 to 5 minutes and a volume of between 100 ml and a litre is administered depending upon the relative strength of the patient.

Once administration has finished and typically after a further period of 5-20, e.g. 10 mins, the guide wire 4 is replaced down the central lumen 12, the balloon 8 is deflated by withdrawing saline therefrom. This allows blood and some of the introduced liquid to flow to the heart 24. The catheter 2 is then removed by sliding it over the guide wire 4 and finally the guide wire 4 is removed.

Thus in accordance with the described apparatus and methods, an improved method of gene therapy exhibiting

significantly higher transfection efficiencies in hepatic liver cells is disclosed.

A further embodiment of the invention is shown in Fig. 7. In this embodiment, the catheter 36 comprises three lumens. In addition to a central guide wire lumen 38, there are upper and lower side lumens 40, 42. The lower side lumen 40 communicates with a pair of axially spaced balloons 44, 46 by means of corresponding side apertures 48, 50. The upper side lumen 42 opens out radially in a series of side apertures 52 located axially between the two balloons 44, 46.

Use of the catheter 36 shown in Fig. 7 is similar to the previous embodiment except that since the nucleic acid is not administered through the guide wire lumen 38, there is no need to withdraw the guide wire (not shown for clarity) during the procedure. Furthermore, the provision of two balloons 44, 46 allows a section of the hepatic vein to be fluidically isolated both upstream and downstream which means that the gene delivery is not affected by blood flow at all and may mean that a higher administration pressure can safely be used as compared to the previous embodiment.

It will be appreciated by those skilled in the art that only certain preferred embodiments of the invention have been described and that there are many variations and modifications possible within the scope of the invention. For example, a centrally guided catheter is not essential and for example a monorail catheter could be used instead. It is also envisaged that the cells undergoing the described therapy may be subjected to ultrasound or other suitable form of radiation in order to enhance the transfection thereof by the nucleic acid. An ultrasonic vibrator e.g. a piezo-electric oscillator could be provided on the catheter for this purpose.

The invention is further described in the following Examples:

Example 1

The following protocol was performed on 2 pigs of around 40 kg.

The pigs were put under general anaesthetic. A catheter was introduced in the neck vein (external jugular). The catheter had 2 channels; one central channel that can carry an introducer (e.g. a guide wire) and another that can be used to inflate a balloon.

The catheter was pushed down from the neck veins under image intensifier to the superior vena cava, right heart, supra-hepatic vena cava until it reached one of the 3 hepatic veins. For the purpose of this experiment the left hepatic vein is the most suitable.

It was introduced until the catheter did not advance any further.

The balloon was then inflated in order to close completely the lumen of the hepatic vein.

Then the introducer was removed and the nucleic acid injected fast, within a minute or two, under pressure. A volume of 500-1000 ml was injected.

The balloon was kept inflated for about 10 minutes, then deflated slowly and the catheter removed.

The anaesthetic was then discontinued and the animal was recovered. Serial blood tests were performed for 3 weeks to check on any toxicity, liver damage as well as gene expression.

These experiments have shown that this technique was safe. The liver function test remained normal and the animal remained in good health. Significant gene expression was observed.

Example 2

In this example the plasmid pDERM II expressing rat TPO (thrombopoietin) under the control of a liver specific promoter was injected into the hepatic vein of rats after inferior vena cava (IVC) occlusion and intravenously into the tail vein of rats (controls). 400 g rats were injected with 100 µg of plasmid. The IVC was clamped just above or in the junction with hepatic veins.

TPO is normally produced in the liver and acts on the bone marrow where it stimulates production of platelets by megakaryocytes. The count of platelets (PLT) and white blood cells (WBC) in 1 ml of blood in the systemic circulation were measured in 7 rats and the mean values for each group calculated. The results are shown in Table 1 below, all values are in thousands.

Table 1

Day 7	Controls		pDERM TPO	
	PLT	*WBC*	*PLT*	*WBC*
	1239	5,5	1416	7,8
	895	6,7	1388	7,9
	826	6,8	1449	7,4
			1411	7,4
	987	6	1416	7,6

These results show that levels of TPO, i.e. plasmid transfection efficiency, are greater where hydrodynamic injection into the hepatic vein is used.

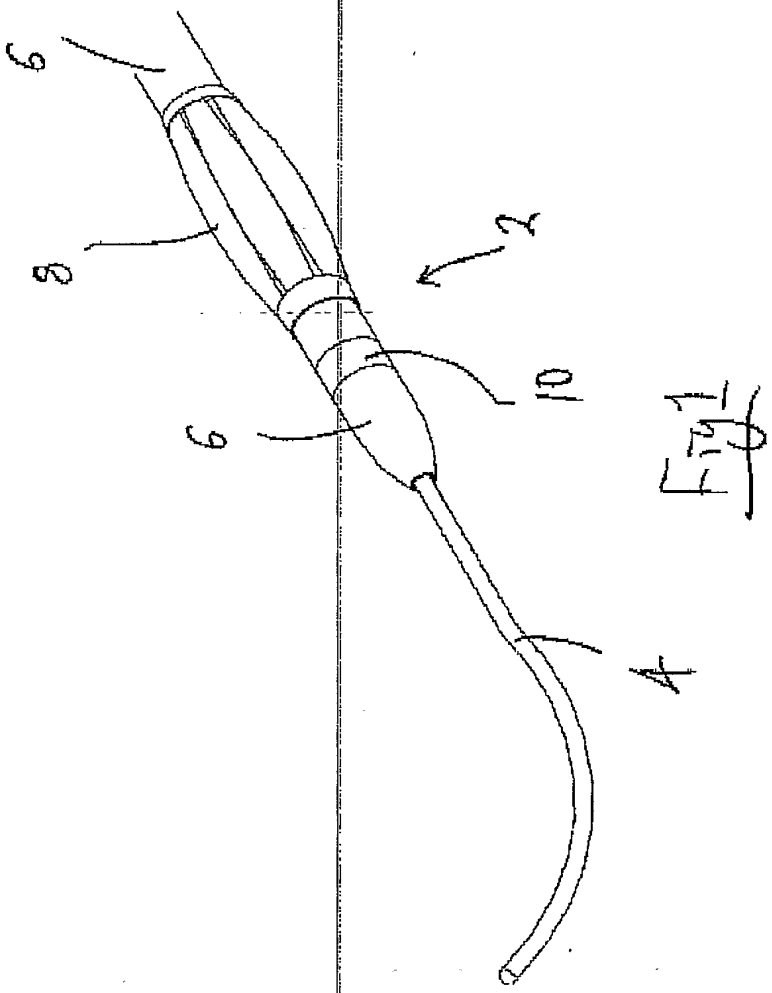

Fig 1

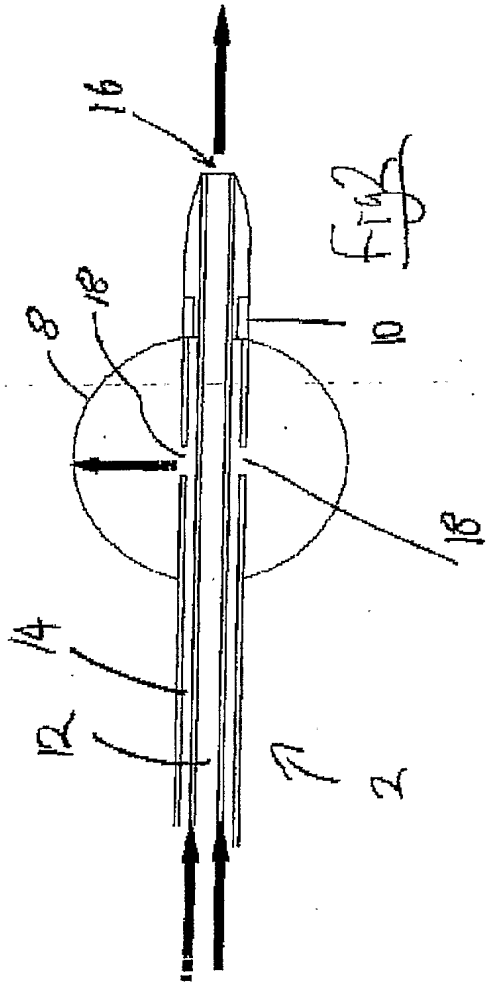

Fig 2

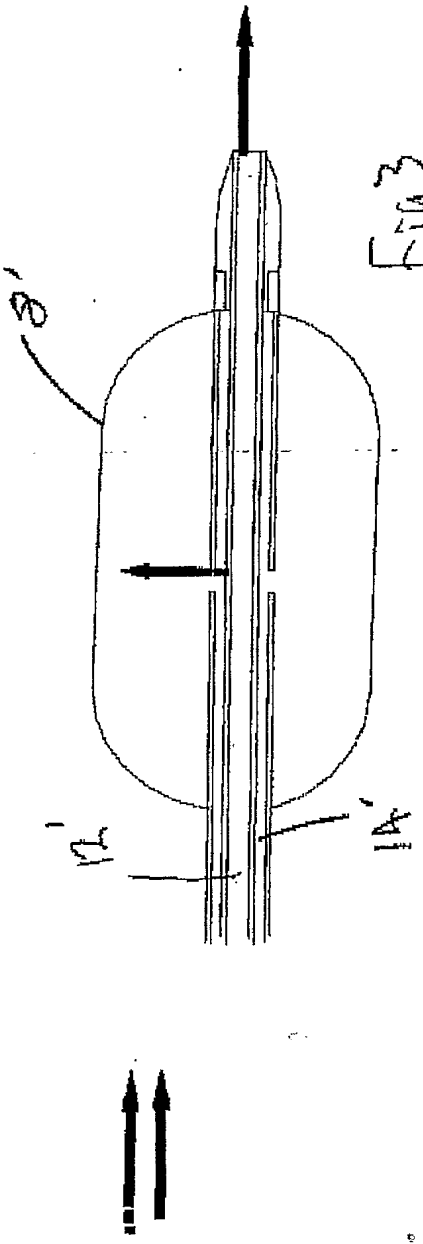

Fig 3

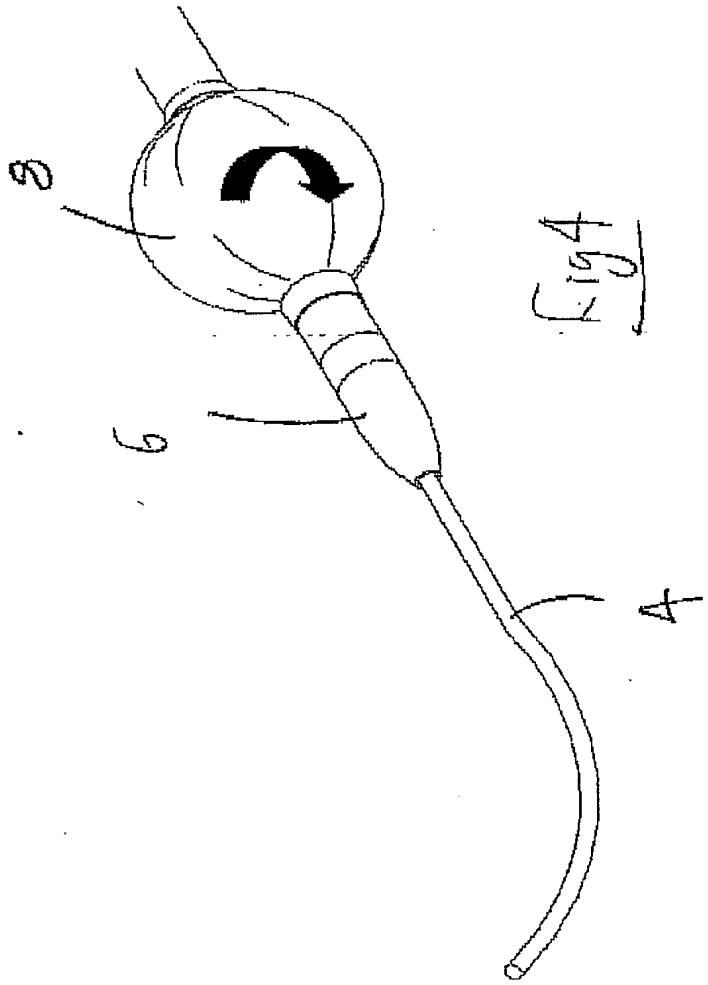

Fig 4

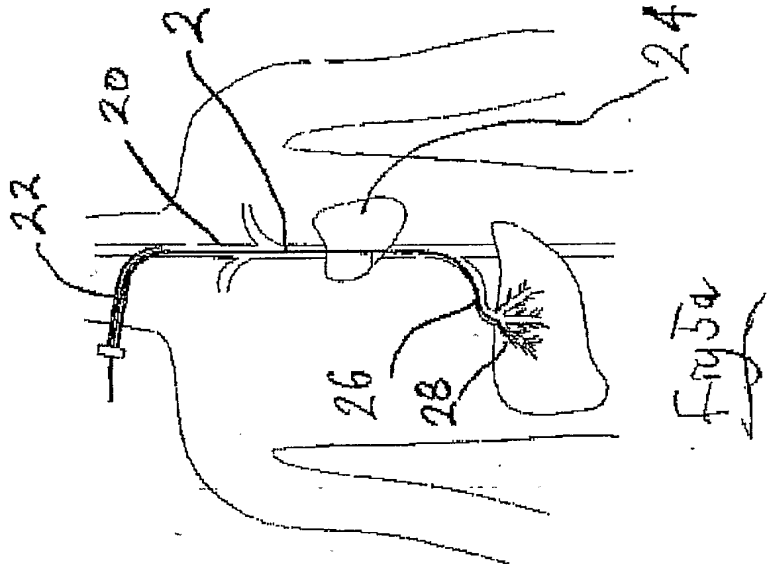

Fig 5a

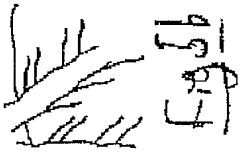

Fig 5b

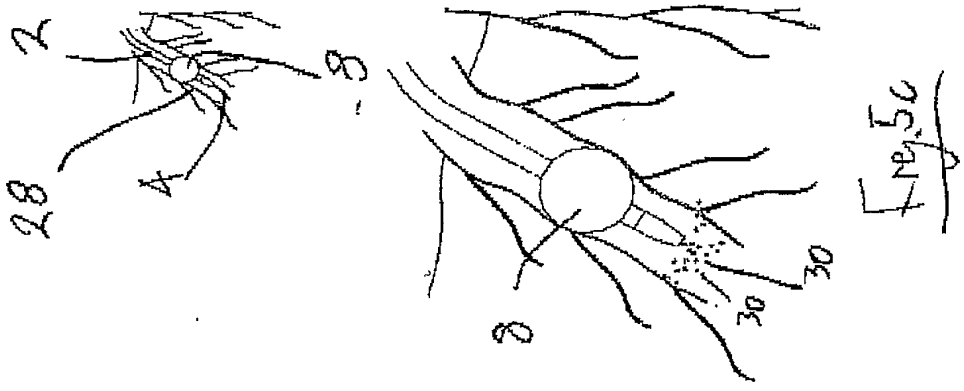

Fig 5c

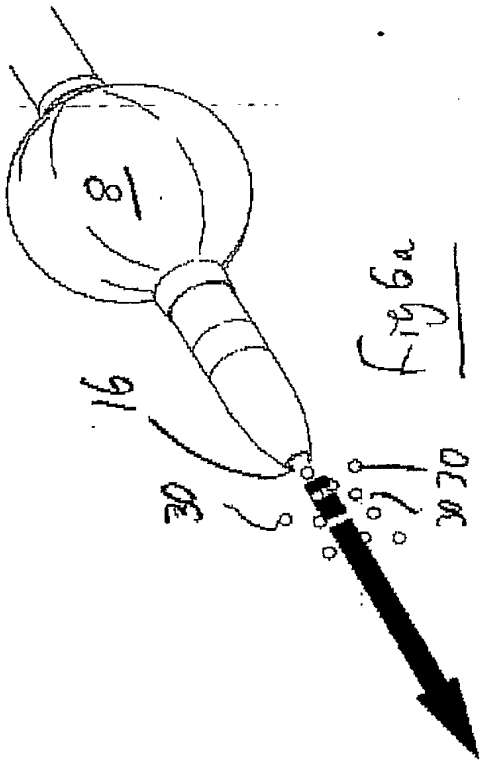

Fig 6a

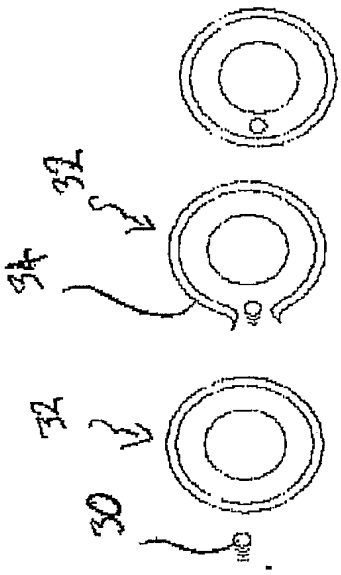

Fig 6b

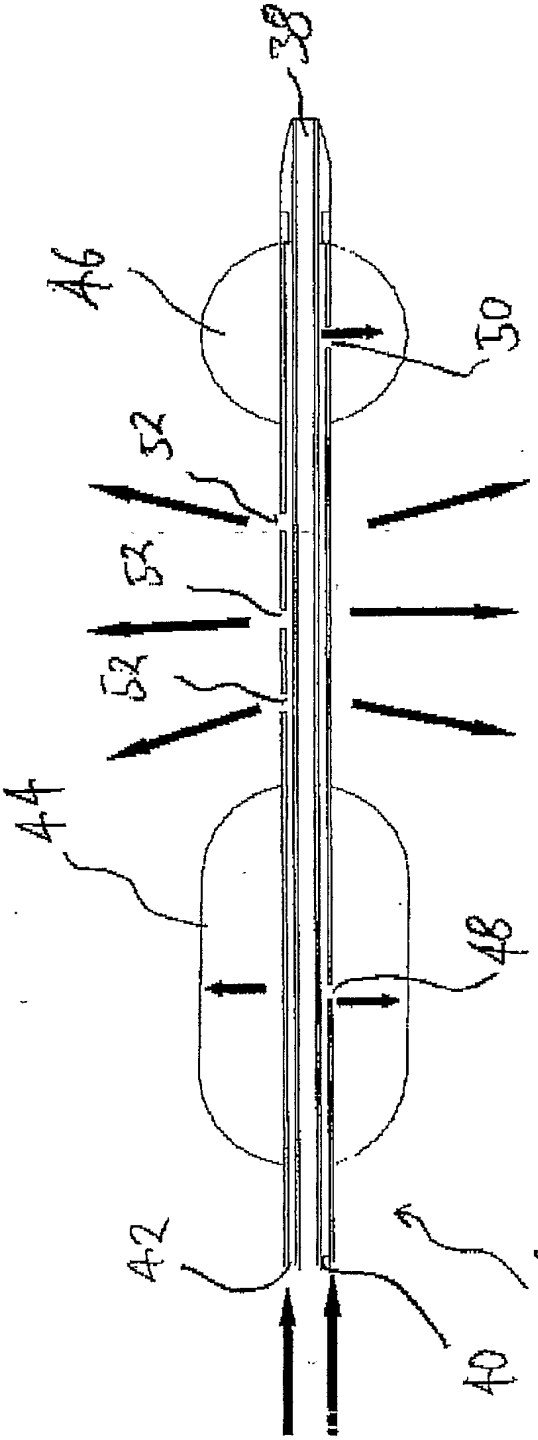

Fig 7